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(57) Abstract

The subject invention provides novel peptides and the use of these peptides in the treatment of various diseases and conditions. The novel peptides specifically bind to undetermined and determined targets in various organs and in lymphocytes. The subject invention also provides a method for the identification of a peptide by applying peptide library methodology *ex vivo* to perfused organs.

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ISOLATION OF TISSUE SPECIFIC PEPTIDE LIGANDS AND THEIR USE FOR TARGETING PHARMACEUTICALS TO ORGANS

This application claims priority of U.S. Serial No. 08/810,074, filed March 4, 1997, and U.S. provisional application No. 60/039,509, filed March 4, 1997.

BACKGROUND OF THE INVENTION

There is a need in the pharmaceutical industry for pharmaceutical agents that can be targeted to specific organs, and thus provide local drug delivery.

Advantages of local drug delivery are the lowering of the amount of drug needed to achieve therapeutic efficacy and the minimizing of undesired side effects.

Currently, the main approach to tissue specific targeting is either to infuse the drug through a catheter or a balloon (PTCA) to a site of the vasculature, or through linking of a drug to a protein ligand with affinity for a predetermined target.

For example, full-size monoclonal antibodies against predetermined cell surface antigens have been generated in order to produce cell targeting ligands. However, the complexity of isolating a specific antibody and the size of such antibody are severe limitations to their use as cell targeting ligands.

The use of a phage peptide display library facilitates an alternative means of producing unique ligands for targeting to specific, yet unrecognized cell (undetermined) surface moieties. Phage libraries have been used to select random peptides that bind to isolated pre-determined target proteins such as antibodies, hormone receptors, and the erythropoietin receptor.

Unlike the use of a known ligand, the process of phage selection from a random peptide library does not require prior knowledge of the target cell or its receptors. This approach also has the advantage that molecular recognition and ligand selection are not dependent on the immunogenicity of the candidate target protein, as required in the monoclonal antibody approach.

Pasqualini and Ruoslahti (Nature 380: 364-366, 1996) injected phage libraries intravenously into mice and subsequently rescued the phage from individual organs. Peptides capable of mediating selective localization of phage to brain and kidney blood vessels were identified and were shown to exhibit up to 13-fold higher sensitivity for these mouse organs. One of the peptides displayed by the brain-localized phage was chemically synthesized and shown to specifically inhibit the localization of the homologous phage into the brain. When coated onto glutaraldehyde fixed red blood cells, the peptide caused selective localization of intravenously injected red blood cells into the brain of the mouse.

The subject invention discloses the use of phage display peptide (epitope) libraries to identify peptides useful as ligands for targeting drugs, cells or genes to specific human tissue and various human organs, where the specific receptor is not predetermined. In addition, some experiments were carried out using predetermined targets.

The novelty of the subject approach is *inter alia* the application of the peptide library methodology to isolated perfused human tissues.

A phage library is included in the organ perfusion fluid, and after *ex vivo* organ perfusion, phages are extracted from the human tissue, amplified and the displayed peptide sequence is determined. This *ex vivo* approach is applied to

human organs such as placenta, umbilical cord artery and vein as well as blood vessels removed during surgery. This approach is further applied to diseased tissue removed during surgery and to organs such as kidney, heart and liver available following transplantation procedures.

The endothelium which lines the inner surface of blood vessels expresses multiple surface proteins and receptors for diverse types of ligands. Endothelial cells, derived from different tissues or even from veins and arteries of the same tissue, have been shown to be phenotypically and functionally distinct. The unique distinctive, characteristic surface proteins and receptors expressed by endothelial cells of the various tissues are exploited to discover novel, defined peptide ligands which are subsequently linked to drugs or radioactive isotopes for targeting to the desired tissue.

The subject invention provides a parallel approach to obtain unique, yet undetermined targets characteristic of lymphatic cells derived from different diseases, such as leukemia and autoimmune diseases. Since lymphocytes are obtained in suspension, biopanning is carried out by mixing a phage library with the lymphocyte cell suspension, followed by washing with several buffer solutions. Biopanning is repeated several times following amplification of selected phage population.

The peptide sequences of the subject invention, specific for different human organs and tissue cells are linked to various pharmaceutical agents to form drug-peptide conjugates and to radioactive isotopes for diagnostic and therapeutic purposes.

Summary of the Invention

The subject invention provides novel peptides and the use of these peptides in the treatment of various diseases and conditions. The novel peptides specifically bind to undetermined targets (and some determined targets) in various organs and in lymphocytes.

The subject invention provides for compositions comprising novel peptides and a pharmaceutical agent linked to the peptide, wherein the pharmaceutical agent is a polypeptide and is linked to the peptide by a peptide linkage. The pharmaceutical agent may also be a toxin, an anti-cancer agent, an anti-angiogenic compound, a cardiovascular agent, an agent used in a neurological disorder, a liver disease agent, a kidney disease agent or a radioisotope.

The subject invention provides for a method for the identification of a peptide comprising incubating a phage display peptide library with an isolated organ; washing the isolated organ to remove unbound phages; eluting bound phage from the isolated organ; amplifying the resulting bound phage; and determining the displayed peptide sequence of the bound phage so as to identify the peptide.

The organ may be an artery, a vein, placenta, tumor tissue, kidney, heart, liver, or central nervous system. The organ may also be a perfused organ.

The phage display library may be a 15-mer library or a 6-mer library.

The elution medium may be a compound selected from acid, urea, Octyl, trypsin or tween.

The subject invention also provides for a method of producing the novel peptides comprising identifying the peptide as described above; and synthesizing the peptide by joining the amino acids of the peptide in the proper order.

The subject invention additionally provides an imaging agent which comprises a peptide of the subject invention with an imageable marker. Such an imaging agent may be used for diagnostic purposes.

The subject invention further provides a composition comprising an effective imaging amount of an imaging agent of the invention and a physiologically acceptable carrier.

The subject invention also encompasses a composition comprising an effective imaging amount of an imaging agent of the invention, a pharmaceutical agent linked thereto and a physiologically acceptable carrier.

The marker may be a radioactive isotope, an element which is opaque to X-rays or a paramagnetic ion.

The radioactive isotope may be indium-111, technetium-99, iodine-123, iodine-125, iodine-131, krypton-81m, xenon-33 or gallium-67.

The subject invention also provides for a method for imaging an organ comprising contacting the organ to be imaged with an imaging agent under conditions such that the imaging agent binds to the organ; imaging bound imaging agent; and thereby imaging the organ.

The subject invention also provides for a method of treating an organ *in vivo* comprising contacting the organ to be treated with a composition under conditions such that the composition binds to the organ; and thereby treating the organ.

Brief Description of the Figure

Figure 1: Organ distribution of selected phages

Clone KSC#3 (KSCR3#3) is a phage clone that was enriched on Kaposi Sarcoma cells after three rounds of biopanning in culture. Clone R4B*#1 (TUV-R4B*#1) is a phage clone that was enriched on umbilical vein and artery. Clone #P13 (R6P#13) is a sporadic non-enriched phage clone as a negative control. The *in vivo* binding of these three clones to tumor tissue and brain tissue was compared.

Detailed Description of the Invention

Phage display is a technique in which a peptide, antibody or protein is expressed on the surface of a bacteriophage, while the DNA encoding the displayed protein resides within the phage virion. A phage display peptide library (also termed phage peptide library or phage display library or phage library or peptide library) is constructed wherein the virions display a wide range of protein residues of specific lengths. This technology, known to one skilled in the art, is more specifically described in the following publications: Smith (1985) Science 228: 1315, Scott et al. (1990), Science 249: 386-390, Cwirla et al. (1990), P.N.A.S. 87: 6378-6382; Devlin et al. (1990), Science 249: 404-406, U.S. Patent Nos 5,427,908, 5,432,018, 5,223,409 and 5,403,484.

Biopanning is a procedure comprising many steps, one of which is selection; biopanning is carried out by incubating phages displaying protein ligand variants (a phage display library) with a target, washing away unbound phage and specifically eluting the bound phage. The eluted phage is amplified and taken through additional cycles of binding and amplification which enrich the pool of eluted specific sequences in favor of the best binding peptide bearing phages. After several rounds, individual phages are characterized, and the sequence of the peptides displayed is determined by sequencing of the corresponding DNA of the phage virion. A peptide obtained in this manner may be

called a "lead-compound".

One way of obtaining a peptide with a higher affinity relative to a lead-compound is to construct an extension phage display peptide library based on a core amino acid sequence of the lead-compound. In such an extension library, random amino acids are added to each side of the core sequence.

An additional way to obtain a peptide with a higher affinity relative to a lead-compound is the construction of a phagemid display mutagenesis library. In such a library, oligonucleotides are synthesized so that each amino acid of the core sequence is independently substituted by any other amino acid.

The subject invention provides a polypeptide which comprises a peptide of the subject invention which corresponds to a peptide displayed on a phage virion and wherein both the polypeptide and the peptide have the same biological activity.

In one embodiment, a peptide of the invention preferably has less than 50 amino acids but more than 5 amino acids.

In another embodiment, a peptide of the subject invention comprises 6-15 amino acids.

In another embodiment, to a peptide of the subject invention, 20-30 amino acids are added either to the C-terminus or the N-terminus of the peptide while the peptide maintains its biological activity.

In another embodiment of the subject invention, several amino acids are added either to the C-terminus or the N-terminus or to both termini of the peptide while maintaining the biological activity of the peptide. In this embodiment, 1-5 amino acids are preferably added to each terminus.

In an additional embodiment, a Fv fragment of a human antibody of about 100 amino acids is displayed on the N-terminus of pIII of M13 bacteriophage. In this embodiment, the peptide of the subject invention is about 100 amino acids.

Cancer tissue as used herein may be obtained from any form of cancer such as carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma or melanoma.

Diseased tissue as used herein may be obtained from any diseased organ such as liver, kidney, lung, heart, ovary, colon and so forth. The organ may be diseased as a result of an autoimmune disorder. The organ may be diseased as a result of any other disease, such as cardiovascular disease or cancer.

A neurologic disorder as used herein encompasses any neurologic disorder as defined and described in "The Merck Manual", sixteenth edition (1992). For example, muscular dystrophy, myasthenia gravis, multiple sclerosis, Alzheimer's disease, neuropathy, Parkinson's disease and amyotrophic lateral sclerosis (Lou Gehrig's disease) are neurologic disorders.

A vein as used herein may originate from any tissue. An example of a vein is safenal vein or femoral vein.

An artery as used herein may originate from any tissue, e.g. radial artery, coronary artery, mammary artery and so forth.

A peptide of the subject invention may be administered to a patient, alone, radiolabeled, linked to a pharmaceutical agent (drug), or in the form of a peptidomimetic.

The mode of administration of a peptide of the subject invention is intravenous, intramuscular, subcutaneous, topical, intratracheal, intrathecal, intraperitoneal,

rectal, vaginal or intrapleural.

The pharmaceutical agent may *inter alia* be a radioactive label (radio-isotope).

If the peptide or the peptide-drug combination is administered orally, it is administered in the form of a tablet, a pill or a capsule.

The compositions comprising the peptides produced in accordance with the invention may comprise conventional pharmaceutically acceptable diluents or carriers. Tablets, pills and capsules may include conventional excipients such as lactose, starch and magnesium stearate. Suppositories may include excipients such as waxes and glycerol. Injectable solutions comprise sterile pyrogen-free media such as saline and may include buffering agents, stabilizing agents or preservatives. Conventional enteric coatings may also be used.

Compositions for topical administration may be in the form of creams, ointments, lotions, solutions or gels.

The mode of administration of the peptide or drug-peptide linkage is a solid dosage form, a liquid dosage form, or a sustained-release formulation.

The subject invention provides peptides comprising amino acids having the following sequences:

Gly Arg Gln His Phe Phe Leu Ala Glu Gly Arg Ser Phe Tyr Phe;

Ser His Val Pro Pro Ile Phe Asn Asp Val Tyr Trp Ile Ala Phe;

Val Pro Pro Ile Phe Asn Asp Val Tyr Trp Ile Ala Phe;

His Thr Phe Phe Leu Pro Gly Cys Ala Gly His Cys Ile Asp Ala;

58. A peptide of claim 1 comprising amino acids having the sequence Val Ser Asp Arg Arg Gln Asn Val

59. A peptide according to claim 58 having the amino acid sequence Val Ser Asp Arg Arg Gln Asn Val

60. A peptide of claim 1 comprising amino acids having the sequence Ser Lys Ser Pro

61. A peptide according to claim 60 having the amino acid sequence Ser Lys Ser Pro

62. A peptide of claim 1 comprising amino acids having the sequence Gly Thr Leu Asn Gln Cys Gly Arg Ile Asn

63. A peptide according to claim 62 having amino acid sequence Gly Thr Leu Asn Gln Cys Gly Arg Ile Asn

64. A peptide of claim 1 comprising amino acids having the sequence Cys Ala Val Glu Ala Ala Gly Pro Val Arg Val Leu

65. A peptide according to claim 64 having amino acid sequence Cys Ala Val Glu Ala Ala Gly Pro Val Arg Val Leu

66. A peptide of claim 1 comprising amino acids having the sequence Ser Gly Ser Leu Gly Arg Ser Leu Glu

67. A peptide according to claim 66 having amino acid sequence Ser Gly Ser Leu Gly Arg Ser Leu Glu

68. A peptide of claim 1 comprising amino acids having the sequence Thr Gly Asp Glu

69. A peptide according to claim 68 having amino acid sequence Thr Gly Asp Glu

70. A peptide of claim 1 comprising amino acids having the sequence Phe Lys Ala Ser Arg His Ser

71. A peptide according to claim 70 having amino acid sequence Phe Lys Ala Ser Arg His Ser

72. A peptide of claim 1 comprising amino acids having the sequence Ile His Met Arg Ala

73. A peptide according to claim 72 having amino acid sequence Ile His Met Arg Ala

74. A peptide of claim 1 comprising amino acids having the sequence Lys Asn Ala Asn

75. A peptide according to claim 74 having amino acid sequence Lys Asn Ala Asn

76. A peptide of claim 1 comprising amino acids having the sequence Met Arg Ala Pro Val Ile

77. A peptide according to claim 76 having amino acid sequence Met Arg Ala Pro Val Ile

78. A peptide of claim 1 comprising amino acids having the sequence Gly Ile Lys Gly Leu Asp Glu

79. A peptide according to claim 78 having amino acid sequence Gly Ile Lys Gly Leu Asp Glu

80. A peptide of claim 1 comprising amino acids having the sequence Cys Lys Trp Glu Lys Arg

81. A peptide according to claim 80 having amino acid sequence Cys Lys Trp Glu Lys Arg

82. A peptide of claim 1 comprising amino acids having the sequence Ala Arg Leu Ser Pro Thr Met Val His

Pro Asn Gly Ala Gln Pro

83. A peptide according to claim 82 having amino acid sequence Ala Arg Leu Ser Pro Thr Met Val His Pro Asn Gly Ala Gln Pro

84. A peptide of claim 1 comprising amino acids having the sequence Ala Leu Gly Gly Phe Arg Pro Phe Trp Ser Tyr Gly Gly Leu Ser

85. A peptide according to claim 84 having amino acid sequence Ala Leu Gly Gly Phe Arg Pro Phe Trp Ser Tyr Gly Gly Leu Ser

86. A peptide of claim 1 comprising amino acids having the sequence Met Gly Ala Asp Asp Ala Pro His Tyr Trp His Pro Val Trp Thr

87. A peptide according to claim 86 having amino acid sequence Met Gly Ala Asp Asp Ala Pro His Tyr Trp His Pro Val Trp Thr

88. A peptide of claim 1 comprising amino acids having the sequence Cys Thr Arg Leu Gly Ala Ala Ala Gly Arg Cys Asp Val Gly Leu

89. A peptide according to claim 88 having amino acid sequence Cys Thr Arg Leu Gly Ala Ala Ala Gly Arg Cys Asp Val Gly Leu

90. A peptide of claim 1 comprising amino acids having the sequence Arg Leu Phe Met Leu Gly

91. A peptide according to claim 90 having amino acid sequence Arg Leu Phe Met Leu Gly

92. A peptide of claim 1 comprising amino acids having the sequence Pro Ile Trp His Gly Asp Ser Gly Val

Tyr Ser Ser Phe Phe Pro

93. A peptide according to claim 92 having amino acid sequence Pro Ile Trp His Gly Asp Ser Gly Val Tyr Ser Ser Phe Phe Pro

94. A peptide of claim 1 comprising amino acids having the sequence Arg Asn Thr Leu Pro His Phe Ser Phe Gly Pro Arg Leu Tyr Arg

95. A peptide according to claim 94 having amino acid sequence Arg Asn Thr Leu Pro His Phe Ser Phe Gly Pro Arg Leu Tyr Arg

96. A peptide of claim 1 comprising amino acids having the sequence Gly Leu Ser Asp Gly pro Tyr Tyr Ser Phe Ser Leu Phe Arg Phe

97. A peptide according to claim 96 having amino acid sequence Gly Leu Ser Asp Gly pro Tyr Tyr Ser Phe Ser Leu Phe Arg Phe

98. A peptide of claim 1 comprising amino acids having the sequence Gly Gly Ala Ala Gly Gly Tyr Leu Arg Val Phe Ala Gly Val Arg

99. A peptide according to claim 98 having amino acid sequence Gly Gly Ala Ala Gly Gly Tyr Leu Arg Val Phe Ala Gly Val Arg

100. A peptide of claim 1 comprising amino acids having the sequence Gly Tyr Asp Cys Trp Asp Cys Pro Phe Ser Phe Arg Gly Ser Val

101. A peptide according to claim 100 having amino acid sequence Gly Tyr Asp Cys Trp Asp Cys Pro Phe Ser Phe Arg Gly Ser Val

102. A peptide of claim 1 comprising amino acids having the sequence Ser Asn Leu Asn Arg Phe Val Phe Ala Phe Trp Asp Gly Pro Ala

103. A peptide according to claim 102 having amino acid sequence Ser Asn Leu Asn Arg Phe Val Phe Ala Phe Trp Asp Gly Pro Ala

104. A peptide of claim 1 comprising amino acids having the sequence Leu His Gly Phe Ala Ser His Lys Asp Gly Pro Leu Ile Pro Ala

105. A peptide according to claim 104 having amino acid sequence Leu His Gly Phe Ala Ser His Lys Asp Gly Pro Leu Ile Pro Ala

106. A peptide of claim 1 comprising amino acids having the sequence Leu Val Phe Val Lys Asn His Pro Leu Val Pro Phe Gly Ser Pro

107. A peptide according to claim 106 having amino acid sequence Leu Val Phe Val Lys Asn His Pro Leu Val Pro Phe Gly Ser Pro

108. A peptide of claim 1 comprising amino acids having the sequence Ser Lys Arg Ala Asn Gly Phe Arg Gly Val Ser

109. A peptide according to claim 108 having amino acid sequence Ser Lys Arg Ala Asn Gly Phe Arg Gly Val Ser

110. A peptide of claim 1 comprising amino acids having the sequence Ile Lys His Tyr Gly Arg Lys Arg Asn

111. A peptide according to claim 110 having amino acid sequence Ile Lys His Tyr Gly Arg Lys Arg Asn

112. A peptide of claim 1 comprising amino acids having the sequence Val Lys Lys Phe Lys Gly Gly Gln Arg Val

113. A peptide according to claim 110 having amino acid sequence Val Lys Lys Phe Lys Gly Gly Gln Arg Val

114. A composition comprising a peptide according to any of claims 2 to 113 and a pharmaceutical agent linked thereto.

115. A composition according to claim 114 wherein the pharmaceutical agent is a polypeptide and is linked to the peptide by a peptide linkage.

116. A composition according to claim 114 wherein the pharmaceutical agent is a toxin, an anti-cancer agent, an anti-angiogenic compound, a cardiovascular agent, an agent used in a neurological disorder, a liver disease agent, a kidney disease agent or a radioisotope.

117. A composition according to claim 114 wherein the pharmaceutical agent is a recombinant protein.

118. A composition comprising a peptide according to any of claims 2 to 113 and a pharmaceutically acceptable carrier.

119. A composition according to claim 114 which additionally comprises a pharmaceutically acceptable carrier.

120. A chimeric polypeptide comprising a first peptide and a second peptide wherein the first peptide is a peptide of any of claims 2-113.

121. A polypeptide according to claim 120 wherein the

second peptide is a toxin, an anti-cancer agent, an anti-angiogenic compound, a cardiovascular agent, an agent used in a neurological disorder, a liver disease agent or a kidney disease agent.

122. A polypeptide according to claim 120 wherein the second peptide is a recombinant protein.

123. A method for the identification of a peptide which comprises:

a. incubating a phage display peptide library with an isolated organ;

b. washing the isolated organ to remove unbound phages;

c. eluting bound phage from the isolated organ;

d. amplifying the resulting bound phage; and

e. determining the displayed peptide sequence of the bound phage so as to identify the peptide.

124. A method of synthesizing a peptide of any of claims 2-113 which comprises joining the amino acids of the peptide in the proper order.

125. A method of producing a peptide which comprises:

a. identifying the peptide by the method of claim 117; and

b. synthesizing the peptide by joining the amino acids of the peptide in the proper order.

126. A method according to claim 123 wherein the isolated organ is a perfused organ.

127. A method according to claim 123 wherein the isolated organ is an artery, a vein, placenta, tumor tissue, kidney, heart, liver, or central nervous system.

128. A method according to claim 127 wherein the artery is umbilical cord artery, a radial artery, a coronary artery or a mammary artery.

129. A method according to claim 127 wherein the artery is a damaged artery.

130. A method according to claim 129 wherein the damaged artery is a damaged coronary artery.

131. A method according to claim 127 wherein the vein is umbilical cord vein, safenal vein or femoral vein.

132. A method according to claim 123 wherein the phage display peptide library is a 15-mer library.

133. A method according to claim 123 wherein the phage display peptide library is a 6-mer library.

134. A method according to claim 123 wherein the elution medium is a compound selected from acid, urea, Octyl, trypsin or tween.

135. An imaging agent which comprises a peptide of any of claims 2-113 labeled with an imageable marker.

136. A composition comprising an effective imaging amount of the imaging agent of claim 135 and a physiologically acceptable carrier.

137. A composition comprising an effective imaging amount of the imaging agent of claim 135, a pharmaceutical agent linked thereto and a

physiologically acceptable carrier.

138. An agent according to claim 135 wherein the marker is a radioactive isotope, an element which is opaque to X-rays or a paramagnetic ion.

139. An agent of claim 138 wherein the marker is a radioactive isotope.

140. An agent of claim 139 wherein the radioactive isotope is indium-111, technetium-99, iodine-123, iodine-125, iodine-131, krypton-81m, xenon-33 or gallium-67.

141. A method for imaging an organ which comprises:

(i) contacting the organ to be imaged with an imaging agent according to claim 135 under conditions such that the imaging agent binds to the organ ;

(ii) imaging bound imaging agent; and

(iii) thereby imaging the organ.

142. A method according to claim 141 wherein the organ is an artery, a vein, placenta, tumor tissue, kidney, heart or liver.

143. A method according to claim 142 wherein the artery is umbilical cord artery, a radial artery, a coronary artery or a mammary artery.

144. A method according to claim 142 wherein the artery is a damaged artery.

145. A method according to claim 144 wherein the damaged artery is a damaged coronary artery.

146. A method according to claim 142 wherein the vein is umbilical cord vein, safenal vein or femoral vein.

147. A composition according to claim 137 wherein the pharmaceutical agent is a polypeptide and is linked to the imaging agent by a peptide linkage.

148. A composition according to claim 137 wherein the pharmaceutical agent is a toxin, an anti-cancer agent, an anti-angiogenic compound, a cardiovascular agent, an agent used in a neurological disorder, a liver disease agent or a kidney disease agent.

149. A composition according to claim 137 wherein the pharmaceutical agent is a recombinant protein.

150. A method of treating an organ *in vivo* which comprises:

(i) contacting the organ to be treated with a composition according to claim 137 under conditions such that the composition binds to the organ; and

(ii) thereby treating the organ.

151. A method according to claim 150 wherein the organ is an artery, a vein, placenta, tumor tissue, kidney, heart, liver, or central nervous system.

152. A method according to claim 151 wherein the artery is umbilical cord artery, a radial artery, a coronary artery or a mammary artery.

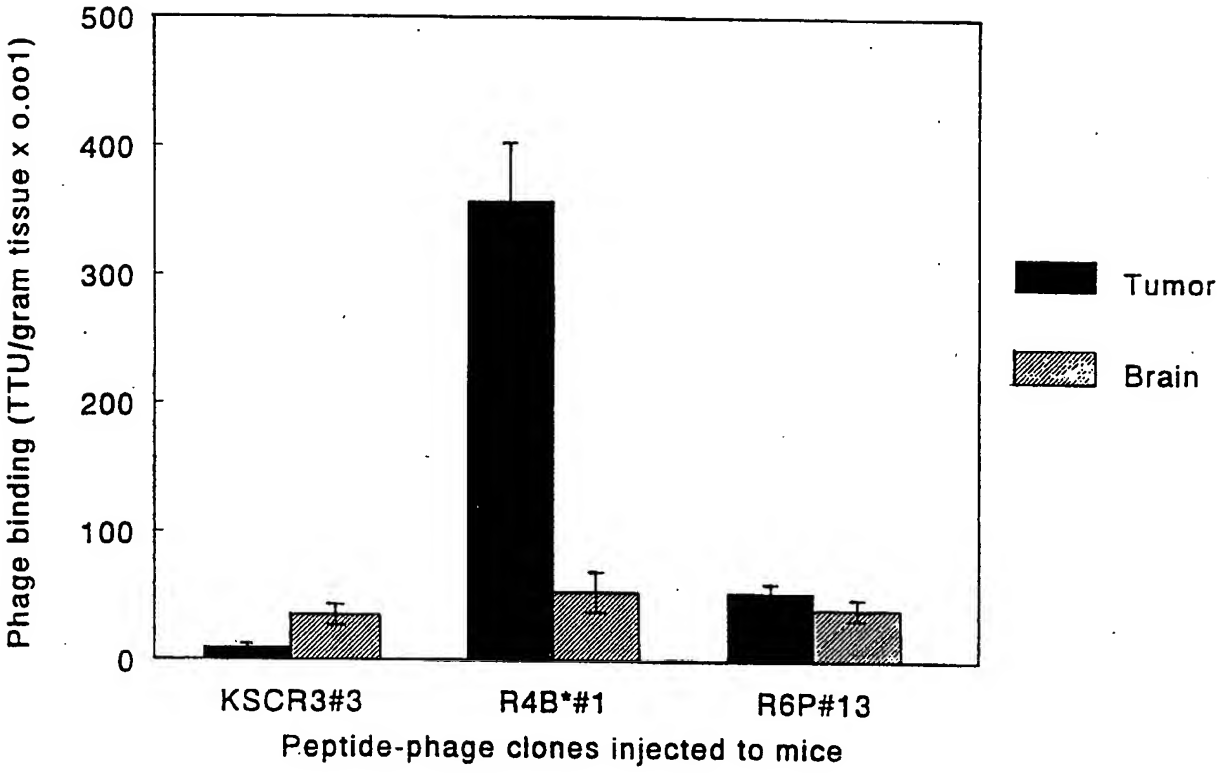
153. A method according to claim 151 wherein the artery is a damaged artery.

154. A method according to claim 153 wherein the damaged

artery is a damaged coronary artery.

155. A method according to claim 151 wherein the vein is umbilical cord vein, safenal vein or femoral vein.

FIGURE 1


500
400
300
200
100
0
Phage binding (TTU/gram tissue x o.oo1)
KSCR3#3
R4B*#1
R6P#13
Peptide-phage clones injected to mice
Tumor
Brain

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/04188

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/00; C07K 7/08, 14/00; G01N 33/543
US CL :435/7.1, 7.21;, 69.7 530/326; 514/14; 436/518

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.21;, 69.7 530/326; 514/14; 436/518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, SCISEARCH. EMBASE. BIOSIS. DERWENT WORLD PATENT, peptide library, coronary arterie, kidney, liver, cell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,510,240 A (LAM et al.) 23 April 1996, see entire article.	1-155
Y	WO 94/26787 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 24 November 1994, see entire article	1-155

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 MAY 1998	Date of mailing of the international search report 08 JUL 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARTHA LUBET Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/04188

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
2

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claim(s) 1-113, 118, 123-136, 138-146, drawn to peptide, method of making the peptide and method of using the peptide to image an organ.
Group II, claim(s) 114-117, 119-122, 137, 147-155, drawn to peptide of Group I linked to a pharmaceutical agent and a method of treating an organ by administering the peptide linked to a pharmaceutical agent.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The special technical feature of Group I is considered to be one of the peptides of Group I.
The special technical feature of Group II is considered to be one of the peptides of Group I linked to a pharmaceutical agent. The peptides of Group I are structurally and functionally distinct from the chimeric peptides of Group II.
A peptide comprising a pharmacuetical agent linked to any of the peptides of Group I has distinct biochemical structure and properties from the peptide itself.
Accordingly, Groups I and II are not so linked by the same or corresponding special technical feature as to form a general inventive concept.

Should Applicant elect Group I, Applicant is required to elect one of the peptides recited in Claims 2-113.

Should Applicant elect Group II, Applicant is required to elect one of the peptides recited in Claims 2-113. After the election of a specific peptide, Applicant is required to elect a specific embodiment of the invention, ie a specific pharmaceutical agent which is linked to the peptide selected from the agents recited in claim 116. For instance, the peptide recited in claim 2 linked to an anti-angiogenic compound.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the peptides recited in claims 2-113 have unique amino acid sequence. Each of the peptides recited in claims 2-113 have distinct biochemical structure and properties, ie binding to particular cell type or elicit peptide specific antibody. Accordingly the peptides recited in claims 2-113 are not so linked by a special technical feature within the meaning of PCT rule 13.2 so as to form a general inventive concept.